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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGY RESEARCH BRANCH

P. O. Box 3391
Orlando, Florida
January 26, 1956

To: C. L. Smith, R. C. Bushland, A. W. Lindquist, W. C. McDuffie,
and C. V. Bowen

From: R. Rodriguez-Torrent

Subject: Special Report on Research

Introduction

I have found it very desirable when certain phases of a research program are terminated or are ready to be applied on a large scale, to write a special report which should contain a critique of the research, recommendations and questions. The monthly and annual reports which you receive are not suited for this type of presentation since we have to adhere in them to a tabulation or an inanimate write-up of facts. Though this may be deemed an appropriate procedure for past research, it does not meet some of the requirements of future research which is mainly based on opinions, subjective but honest appraisal of data, and questions.

I am perfectly aware this type of report is not a common occurrence in your fields of action. I hope herein to give you a birds-eye view of the chemical side of the project. It is not only intended as general information but more so to give you a chance to make suggestions. Many of the suggested ideas may not be appropriate, but just one that works may mean success.

Part I

The Larval-Rearing-Medium

A. The fat problem

No further discussion is deemed necessary on the maximum amount of fat permissible in beef used for artificially growing larvae. These experiments were concluded with the statement that no harmful effects should be experienced when growing larvae with meat from which the excess fat has been trimmed off and complying with a specification of a maximum of 12% fat.

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGY RESEARCH BRANCH

T. O. Box 3381
Gainesville, Florida
January 20, 1956

To: C. E. Smith, E. O. Washburn, A. W. Lindquist, W. C. McCallie,
and G. V. Brown

From: R. H. Hootman or Torrant

Subject: Special Report on Research

Introduction

I have found it very desirable when certain phases of a research pro-
gram are maintained or are ready to be applied on a large scale, to write
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I am particularly aware this type of report is not a common occurrence
in our field of action. I hope herein to give you a different view of
the scientific side of the project. It is not what I intended as a general
information, but more so to give you a chance to take exception. Any
of the material here may not be accurate, but just one that works
very well.

Part I

The Larval-Medium-Medium

The Fat Problem

No further discussion is necessary of the maximum amount
of fat available in beef used for artificial growing larvae. These ex-
periments were conducted with the statement that no artificial fat should
be experienced when growing larvae with meat from which the excess fat has
been trimmed off and containing with a concentration of a maximum of 12% fat.

2-C. L. Smith, R. C. Bushland, A. W. Lindquist, W. C. McDuffie, and
G. V. Bowen-1-26-56

B. The cheaper medium

So far we have not been successful in developing a cheaper medium out of casein, gelatin, and blood. More emphasis will be placed in the future on the development of a cheaper medium and a better utilization of the present one now that two of the problems are out of the way. The cheaper medium approach will consist of the following: (1) Adding fat to the casein-gelatin combinations, (2) adding some meat to the casein and gelatin with the hope the larval, bacterial, and autolytic enzymes start working on the casein and gelatin to make them a suitable food material, (3) use of fresh yeast with and without meat (the yeast is to be obtained from the breweries or bakeries) (4) fish meal and meat combinations. The fish meal is to be partly hydrolyzed.

C. A better use of standard medium

With respect to the better utilization of the present medium it can be stated that our experiments indicate that less standard medium is needed per pan per same number of larvae when using the formulas mentioned below. The four treatments are as follows:

1. Lactose - 0.75%
Sodium nitrite - 1.0 g. per liter water
Sodium nitrate - 2.0 g. per liter water
These pans are inoculated with Lactobacilli
2. Lactose - 0.75%
Formalin - 0.2%
3. Stannic chloride - 2 g. per liter water
Sodium nitrate - 4 g. per liter water
4. Stannic chloride - 2 g. per liter water
Sodium nitrite - 1 g. per liter water
Sodium nitrate - 3 g. per liter water
Lactic acid - 1 cc. per liter water
(Treatments 1 and 2 were suggested by Mr. Brandstein)

These tests, though carried all the way to the size of the large pans, still have to be subjected to a large ^{production} ~~population~~ schedule to prove beyond doubt their advantages and disadvantages. Without any doubt, when properly handled, these four treatments considerably reduce the putrefactive type of decomposition with its ever present obnoxious smell. If accepted, any of these formulations could also be used to further ^{improve} a new medium.

B. The cheaper medium

So far we have not been successful in developing a cheaper medium out of casein, gelatin, and blood. More emphasis will be placed in the future on the development of a cheaper medium and a better utilization of the present one now that two of the problems are out of the way. The cheaper medium approach will consist of the following: (1) Adding fat to the casein-gelatin combinations, (2) adding some meat to the casein and gelatin with the hope the larval, bacterial, and autolytic enzymes start working on the casein and gelatin to make them a suitable food material, (3) use of fresh yeast with and without meat (the yeast is to be obtained from the previous or bacteria) (4) fish meal and meat combinations. The fish meal is to be partly hydrolyzed.

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Sodium nitrate - 1.0 g. per liter water
Sodium nitrate - 2.0 g. per liter water
These pans are inoculated with lactobacilli

2. Lactose - 0.75%
Formalin - 0.2%

3. Stannic chloride - 2 g. per liter water
Sodium nitrate - 1 g. per liter water

4. Stannic chloride - 2 g. per liter water
Sodium nitrate - 1 g. per liter water
Sodium nitrate - 3 g. per liter water
Lactic acid - 1 cc. per liter water

(Treatments 1 and 2 were suggested by Mr. Brandstein)

These tests, though carried all the way to the size of the large pans, still have to be subjected to a large-scale schedule to prove beyond doubt their advantages and disadvantages. Without any doubt, when properly handled, these four treatments considerably reduce the nutritive type of decomposition with its ever present obnoxious smell. If accepted, any of these formulations could also be used to further a new medium.

3-C. L. Smith, R. C. Bushland, A. W. Lindquist, W. C. McDuffie, and
C. V. Bowen-1/26/56

With or without the above formulas, we intend to start experiments towards a better distribution of the medium during the period of larval growth. That is, feed the media to the larvae in increments as needed and not have a pan exposed to enzyme decomposition for 24 hours before the larvae get to run all through it. For example, we could try a system of concentric squares or circles, starting the larvae in the middle and smallest one and then adding fresh medium to the next square when necessary. It seems rather incredible that to grow slightly less than a pound of larvae we should need 7-8 pounds of meat alone, not counting the blood added as a nutrient.

Part II

The Obnoxious Odors

1. Partial list of gases or vapors given off by proteinaceous matter

(1) ammonia, (2) carbon dioxide, (3) hydrogen sulfide
(4) mercaptans (5) skatole (6) indole (7) putrescine
(8) cadaverine

Omitting the use of chemical formulas the origin of each is as follows:

- 1 - For ammonia:

Proteins $\xrightarrow{\text{enzymes}}$ amino acids $\xrightarrow{\text{enzymes}}$
deamination reaction

ammonia + keto acid,
or it may be formed from urea excreted by the larvae,
urea $\xrightarrow{\text{urease}}$ ammonia + carbon dioxide

A possible explanation of the high concentration of ammonia in the rearing cabinets and in the room could be that not only are the larvae excreting urea which is broken down to ammonia, but enzymes are also breaking down the proteins in the meat and blood to give more ammonia by a deamination mechanism. This then becomes a vicious circle, for the alkalinity produced by the ammonia in the media in turn enhances the putrefactive type of enzyme decomposition which needs an alkaline medium to occur at a fast rate. It should be mentioned that ammonia is extremely soluble in water, therefore, unless eliminated plenty of it will dissolve in the water of the medium.

2. Origin of carbon dioxide

Not only from respiration of the larvae, but also from decarboxylation of the amino acids:

Amino acid $\xrightarrow{\text{enzyme}}$ amine and carbon dioxide
decarboxylase

With or without the above formulas, we intend to start experiments towards a better distribution of the medium during the period of larval growth. That is, feed the media to the larvae in increments as needed and not have a pan exposed to enzyme decomposition for 24 hours before the larvae get to run all through it. For example, we could try a system of concentric squares or circles, starting the larvae in the middle and smallest one and then adding fresh medium to the next square when necessary. It seems rather incredible that to grow slightly less than a pound of larvae we should need 7-8 pounds of meat alone, not counting the blood added as a nutrient.

Part II

The Onchocerca Odors

I. Partial list of gases or vapors given off by proteinaceous matter

- (1) ammonia, (2) carbon dioxide, (3) hydrogen sulfide
- (4) mercaptans (5) skatole (6) indole (7) putrescine
- (8) cadaverine

Omitting the use of chemical formulas the origin of each is as follows:

I - For ammonia:

Proteins enzymes → amino acids enzymes → decomposition reaction

ammonia + keto acid,
 or it may be formed from urea excreted by the larvae,
 urea urease → ammonia + carbon dioxide

A possible explanation of the high concentration of ammonia in the rearing cabinets and in the room could be that not only are the larvae excreting urea which is broken down to ammonia, but enzymes are also breaking down the proteins in the meat and blood to give more ammonia by a decomposition mechanism. This then becomes a vicious circle, for the alkalinity produced by the ammonia in the media in turn enhances the putrescine type of enzyme decomposition which needs an alkaline medium to occur at a fast rate. It should be mentioned that ammonia is extremely soluble in water, therefore, unless eliminated plenty of it will dissolve in the water of the medium.

2. Origin of carbon dioxide

Not only from respiration of the larvae, but also from decarboxylation of the amino acids:

Amino acid enzyme → amine and carbon dioxide
 decarboxylase

4-C. L. Smith, R. C. Bushland, A. W. Lindquist, W. C. McDuffie, and
C. V. Bowen-1/26/56

3. For origin of hydrogen sulfide and mercaptans

From amino acids containing sulfur such as cysteine, methionine by both decarboxylation and deamination caused by putrefactive enzymes

4. For origin of skatole and indole

Both are obtained from tryptophane. By a reduction mechanism indole is produced; through an oxidation mechanism skatole is produced.

5. For origin of putrescine and cadaverine

These are produced from the diamino acids; for example, lysine produces when acted on by putrefactive enzymes cadaverine, and arginine produces putrescine.

Except for ammonia and carbon dioxide all the others have highly powerful odors even though found in very small quantities in the air. The odor threshold (concentration below which the odor can not be detected) is given below for a few gases:

Ammonia	- 53 parts per million
Hydrogen sulfide	- 0.18 per parts per million
Ethyl mercaptan	- 0.00026 parts per million

It seems at first glance that activated charcoal is the solution to the problem since it would eliminate ^{most of} these from the cabinets and rooms. However activated charcoal will not pick up ammonia or carbon dioxide, and it has a very low retentivity for sulfides. Nevertheless, if it is used in connection with a suitable system of scrubbing towers which completely eliminate ammonia and reduce the others to very low concentrations, activated charcoal scrubbing is cheap and unsurpassed for air purification. Its real purpose would be to contend with leakage from the cabinets and gaseous infiltrations during transfers.

Chlorine gas could be metered with chlorinators to eliminate the odors if all gases were discharged to the outside, but in our case it is not an acceptable solution. In the first place, the B.T.U's. lost especially in winter, would make conditioning expenses unnecessarily high, and secondly, at the Bithlo plant the odorous gases could be discharged as such to the surroundings since there are no homes in the vicinity. It is to be remembered that chlorine is a very toxic gas and its controlled release into the room is too critical for us to take such chances.

3. For origin of hydrogen sulfide and mercaptans

From amino acids containing sulfur such as cysteine, methionine by both decarboxylation and deamination caused by putrefactive enzymes

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5-C. L. Smith, R. C. Bushland, A. W. Lindquist, W. C. McDuffie, and
C. V. Bowen-1/26/56

Ozone cannot be used to remove these odors for ozone in concentrations above those prevailing in outdoor air is injurious rather than beneficial. In concentrations permissible in occupied spaces (0.01-0.05 p.p.m. parts of air) it does not destroy odors and human beings are injuriously affected by concentrations as low as 1 p.p.m. of air.

An air evacuation system for the rearing cabinets and rooms would be very inefficient in removing these types of odors unless a rather high negative pressure is maintained, thus bringing about that high operating cost already mentioned.

Part III

The removal of the obnoxious odors

Now that the problem has been elucidated it is possible to make attempts at probable solutions. Our experiments indicate that the larvae can live in an atmosphere of hypochlorous acid. We have found that a scrubbing tower filled with calcium hypochlorite will oxidize to inoffensive odors the gases mentioned in part II. The inorganic sulfides are converted to sulfates, the organic sulfides to disulfides, and the amines, putrescine and cadaverine lose their amino groups instantly. We know that skatole and indole are oxidized but whether the change is to an addition product or to an amine oxide nobody knows. Hypochlorous acid is a powerful oxidizing agent and it will oxidize ammonia to chloramine, then some hydrazine can also be formed. Hydrazine is a violent poison, therefore we need to eliminate the ammonia from the gases coming in contact with the hypochlorous acid, or also scrub out the chloramine and hydrazine formed.

It now becomes possible to carry out the final tests in the rearing room at Orlando with the information and data on hand as soon as the equipment arrives. It is evident that a feasible set-up should consist of one or more scrubbing towers for the cabinets, charcoal scrubbers for the room and/or the cabinets, and a bleeder fan to admit fresh air when necessary.

In the annual report I cited the following system: Air from the cabinets goes through 4 scrubbers filled with Raschig rings or Berl saddles. The first scrubber will eliminate ammonia by using a water spray; the second would contain sodium acid sulfate solution to pick up any ammonia left and thus prevent the formation of chloramine and hydrazine; the third would contain calcium hypochlorite to oxidize the sulfides, skatole, indole, putrescine, cadaverine and etc.; the fourth would contain water heated to the desired temperature to put back the lost moisture in the returning air.

Ozone cannot be used to remove these odors for ozone in concentrations above those prevailing in outdoor air is injurious rather than beneficial. In concentrations permissible in occupied spaces (0.01-0.02 p.p.m. parts of air) it does not destroy odors and human beings are injuriously affected by concentrations as low as 1 p.p.m. of air.

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In the annual report I cited the following system: Air from the cabinets goes through a scrubber filled with Rancho Kings or Best saddles. The first scrubber will eliminate ammonia by using a water spray; the second would contain sodium acid sulfate solution to pick up any ammonia left and thus prevent the formation of chloramine and hydrazone; the third would contain calcium hypochlorite to oxidize the sulfides, skatole, indole, putrescine, cadaverine and etc.; the fourth would contain water heated to the desired temperature to put back the lost moisture in the returning air.

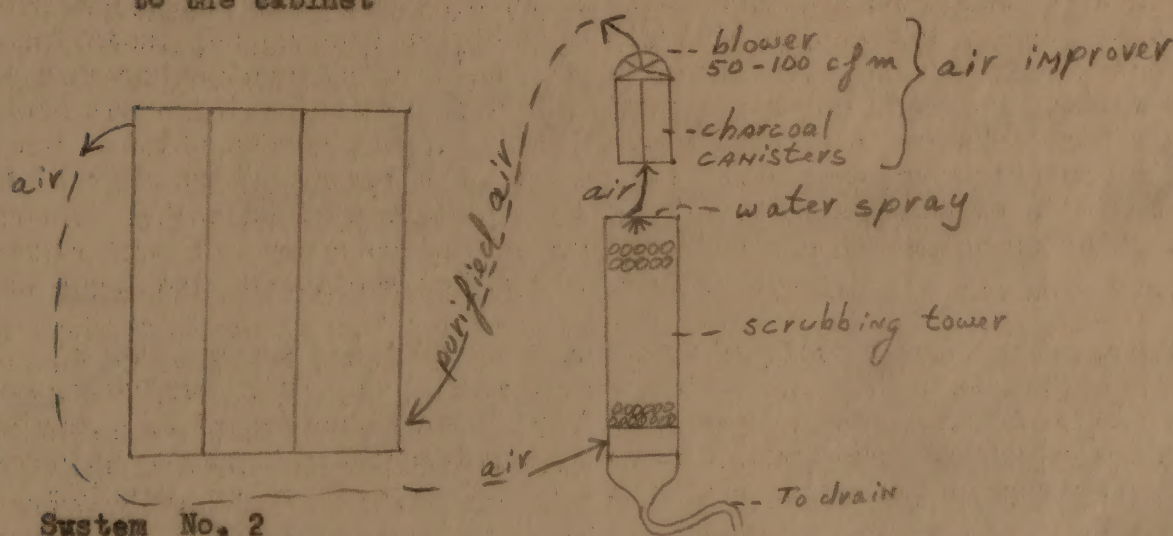
6-C. L. Smith, R. C. Bushland, A. W. Lindquist, W. C. McDuffie, and
C. V. Bowen-1/26/56

In the operation you are planning at Bithlo simple and economical procedures are a necessity. With this in mind information was requested from the Connor Engineering Corporation and Professor Robert Walker of the Chemical Engineering Department, University of Florida. With this additional information it seems that one of several simple systems should give a clean and healthy atmosphere in the rearing room. In all the systems mentioned below a bleeder-opening to admit some fresh air, and activated charcoal in cells or canisters (as shown in the photostats) for the room, are necessities.

System No. 1

	Approx. cost
Components: One scrubbing tower 4 inches to 6 inches diameter, 4 ft. to 6 ft. in length, filled with Berl saddles	\$150.00
Blower - 50-100 cu. ft. per minute	50.00
Dorex Air Improver (containing activated charcoal)	40.00
Approx. total cost	\$240.00

Air from the cabinet is blown upward through a water scrubber, passed to the air improver, and then returned to the cabinet



Components: Same as above, ~~approximate total cost~~ but air is returned to the room, thus creating a negative pressure in the rearing cabinets \$240.00

G. V. Bowen-1/26/56
 G. C. Smith, R. C. Bushland, A. W. Lindquist, W. C. McMillan, and

In the operation you are planning at Mitho simple and economical procedures are a necessity. With this in mind information was requested from the Connor Engineering Corporation and Professor Robert Walker of the Chemical Engineering Department, University of Florida. With this additional information it seems that one of several simple systems should give a clean and healthy atmosphere in the resting room. In all the systems mentioned below a blower-opening to admit some fresh air, and activated charcoal in cells or canisters (as shown in the photostats) for the room, are necessities.

System No. 1

Approx.
 cost

Components: One scrubbing tower 4 inches to 6 inches diameter, 4 ft. to 6 ft. in length, filled with Berl saddles
 Blower - 50-100 cu. ft. per minute
 Dorex Air Improver (containing activated charcoal)
 \$150.00
 50.00
 40.00
 \$240.00 Approx. total cost

Air from the cabinet is blown upward through a water scrubber, passed to the air improver, and then returned to the cabinet



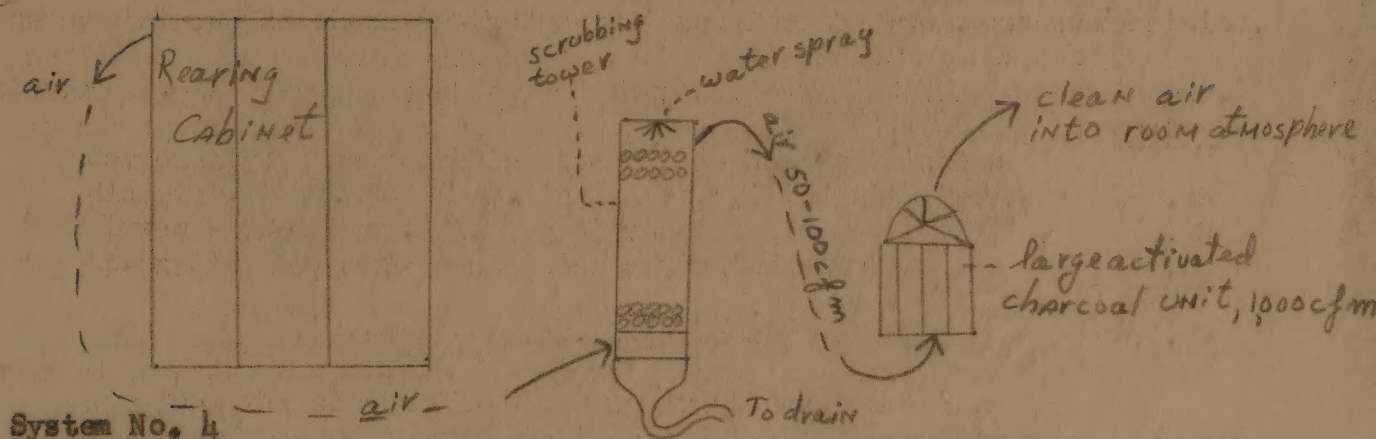
System No. 2

Components: Same as above, approximate total cost \$240.00
 but air is returned to the room, thus creating a negative pressure in the resting cabinets

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C. V. Bowen-1/26/56

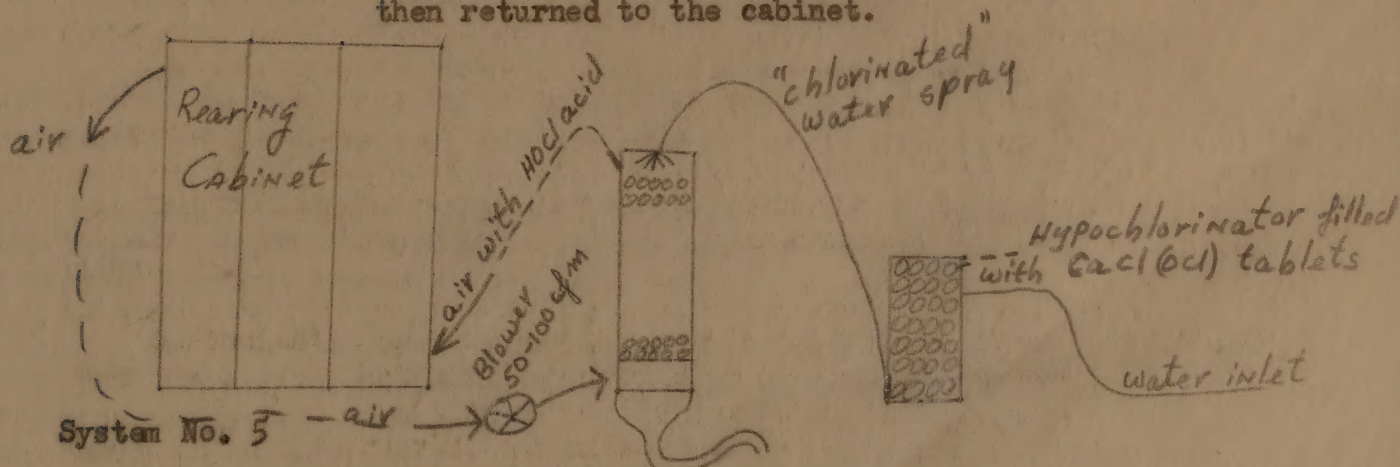
System No. 3

Components: Scrubbing tower \$150.00
Air from the cabinet is passed through
the water scrubber, then passed to the
main activated charcoal unit in the room



Components: Scrubbing tower \$150.00
Blower 50.00
Hypochlorinator
Approximate total cost

Air from the cabinet is blown through a
scrubber containing a hypochlorite spray,
then returned to the cabinet.



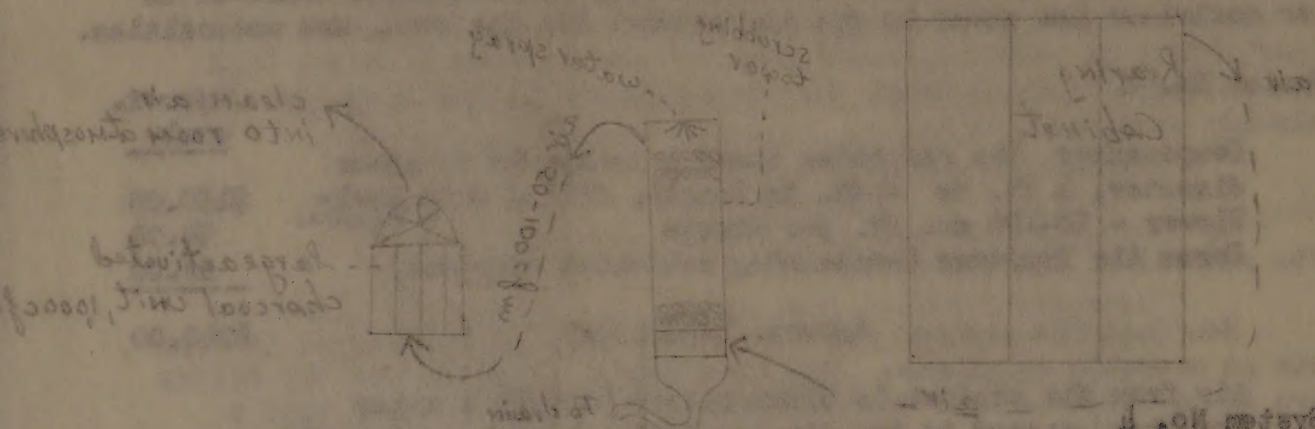
Components: Two scrubbing towers \$350.00
Blower 50.00
Hypochlorinator
Approximate total cost

T-C. L. Smith, R. C. Bushland, A. W. Lindquist, W. C. McDuffie, and
C. V. Bowen-1/20/50

System No. 3

Components: Scrubbing tower
Air from the cabinet is passed through the water scrubber, then passed to the main activated charcoal unit in the room

\$150.00



System No. 4

Components: Scrubbing tower
Blower
Hypochlorinator
Approximate total cost

\$150.00

\$0.00

Air from the cabinet is blown through a scrubber containing a hypochlorite spray, then returned to the cabinet.



System No. 5

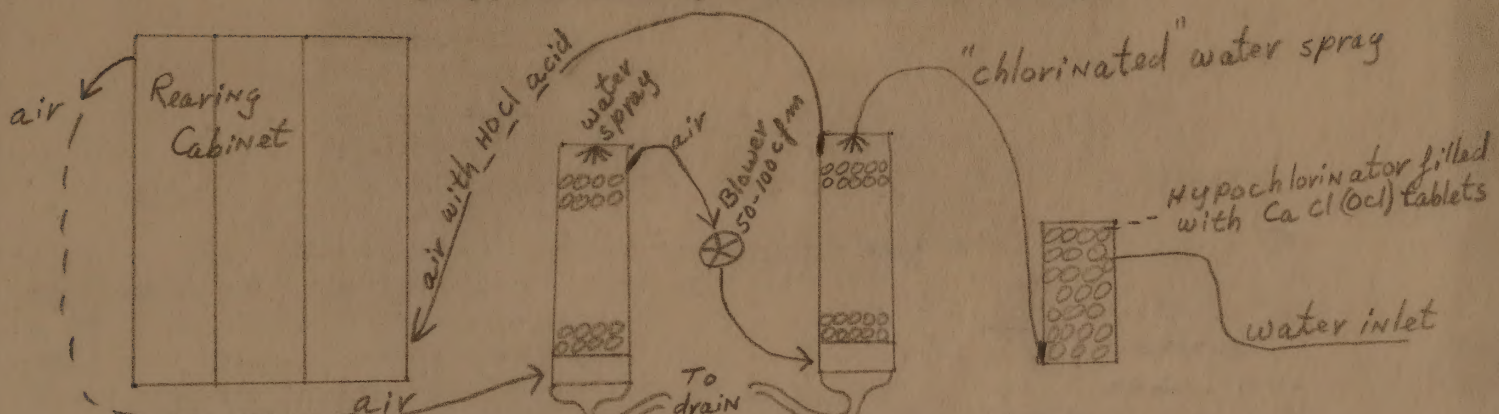
Components: Two scrubbing towers
Blower
Hypochlorinator
Approximate total cost

\$350.00

\$0.00

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C. V. Bowen-1/26/56

Air from the cabinet is blown through a water scrubber to remove ammonia, then through the second scrubber containing the hypochlorite spray, and finally returned to the cabinet.



The total cost for the large rearing room would be approximately broken down as follows:

Bleeder fan	\$ 20.00
Large activated charcoal unit for room	150.00 - 340.00
Equipment for one of the systems mentioned above	300.00
scrubbing	

Enclosed are photostats and pictures pertaining to the above discussion.

Part IV

Recommendations

1. The four treatments to improve the standard medium given under Part I C, should be fully tested by the entomologists under favorable and optimum conditions, both for the larvae and the new medium.

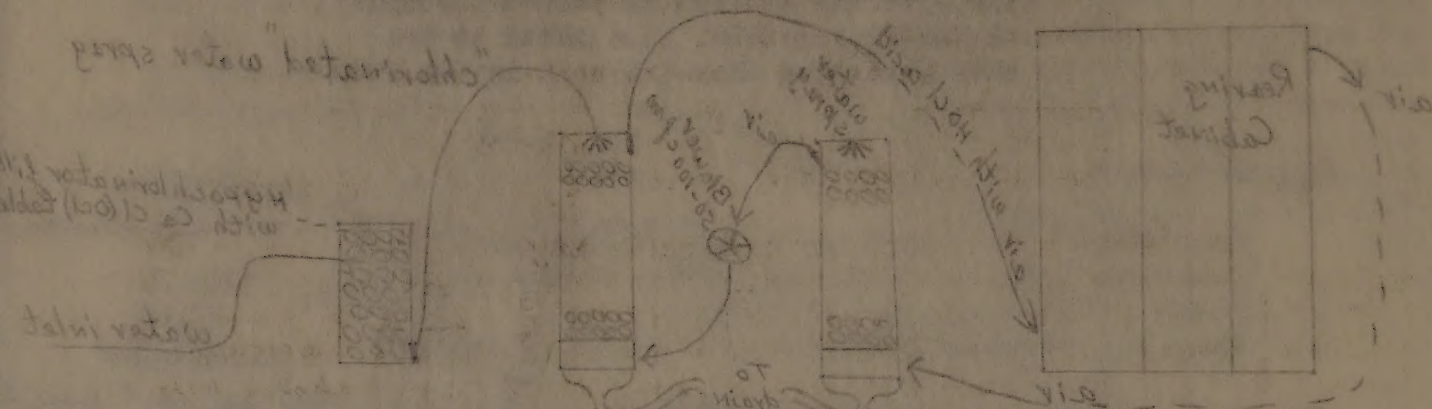
2. The rearing house at Orlando should be scrubbed and painted, after which a set-up of scrubbing towers and activated charcoal units found acceptable should be permanently installed.

3. Since sanitation will be an important factor in the success of the project at Bithlo and will add to the comfort of all concerned, the following points are recommended:

- Chlorox (5 percent) should be added to the vats where the used pans are going to be left soaking.
- Floors should be mopped daily with dilute chlorox solution

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Air from the cabinet is blown through a water scrubber to remove ammonia, then through the second scrubber containing the hypochlorite spray, and finally returned to the cabinet.



The total cost for the large rearing room would be approximately broken down as follows:

Bleeder fan \$ 20.00
Large activated charcoal unit for room 20.00 - 30.00
Equipment for one of the systems mentioned above 300.00

Enclosed are photostats and pictures pertaining to the above discussion.

Part IV

Recommendations

1. The four treatments to improve the standard medium given under Part I C, should be fully tested by the entomologists under favorable and optimum conditions, both for the larvae and the new medium.
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9-C. L. Smith, R. C. Bushland, A. W. Lindquist, W. C. McDuffie, and
C. V. Bowen-1/26/56

- c. Pans taken out of the cabinets for disposal should be immediately sprinkled with a hypochlorite or nitrite solution while still in the large rearing room. (This procedure cuts down the load on the activated charcoal unit).
- d. The drums containing the decomposed media (garbage) should always have at the top a layer of calcium hypochlorite powder (70% available chlorine) and the decomposed media never exposed to the atmosphere.

4. Adequate activated charcoal units should be installed in the wash room, adult room, and the first rearing room where the larvae will be 1 day old. There is no need for scrubbing towers in these rooms since the activated charcoal units will handle the undesirable odors efficiently. The canisters in these units may last up to a year, and replacements cost only a few dollars. For example the unit for the first rearing room will cost only \$39.27.

5. Ammonia and carbon dioxide at least, have to be removed from the large rearing room and proper ventilation sustained. This will be done with suitable scrubbing towers and a bleeder, but for further health insurance and comfort of the employees an adequate activated charcoal unit should be installed.

Part V

Suggestions (These are not directly concerned with my work)

1. A system of portable conveyor belts should be tried at this pilot plant stage for possible use at the much larger plant you have in mind. For example, it will cut handling expenses and in transfers from the rearing room to the washroom avoid spillage of the decomposed media and reduce the time the pans are releasing odors while exposed to the room atmosphere.

2. Thought should be given to calling back Mr. Chester Husman so that he may set up the equipment and I can dedicate more time to the development of a cheaper medium and further improvements in the utilization of the present one.

3. There may be in the near future at Bithlo or at the larger plants you have in mind, a problem in the disposal of the decomposed media because of the large quantities involved. The possibility exists that it can be chemically treated at the plant site to bring about a rapid oxidation or something similar to a sewage treatment process. The dried material produced could be easily carted.

- c. Pans taken out of the cabinets for disposal should be immediately sprinkled with a hypochlorite or nitrite solution while still in the large rearing room. (This procedure cuts down the load on the activated charcoal unit).
- d. The drums containing the decomposed media (garbage) should always have at the top a layer of calcium hypochlorite powder (70% available chlorine) and the decomposed media never exposed to the atmosphere.
- e. Adequate activated charcoal units should be installed in the wash room, adult room, and the first rearing room where the larvae will be 1 day old. There is no need for scrubbing towers in these rooms since the activated charcoal units will handle the undesirable odors efficiently. The canisters in these units may last up to a year, and replacements cost only a few dollars. For example the unit for the first rearing room will cost only \$39.27.
- f. Ammonia and carbon dioxide at least, have to be removed from the large rearing room and proper ventilation maintained. This will be done with suitable scrubbing towers and a blower, but for further health insurance and comfort of the employees an adequate activated charcoal unit should be installed.

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1. A system of portable conveyor belts should be tried at this pilot plant stage for possible use at the much larger plant you have in mind. For example, it will cut handling expenses and in transfers from the rearing room to the washroom avoid spillage of the decomposed media and reduce the time the pans are releasing odors while exposed to the room atmosphere.
2. Thought should be given to calling back Mr. Chester Hansen so that he may set up the equipment and I can dedicate more time to the development of a cheaper medium and further improvements in the utilization of the present one.
3. There may be in the near future at Elkhart or at the larger plants you have in mind, a problem in the disposal of the decomposed media because of the large quantities involved. The possibility exists that it can be chemically treated at the plant site to bring about a rapid oxidation or something similar to a sewage treatment process. The dried material produced could be easily carried.

10-C. L. Smith, R. C. Bushland, A. W. Lindquist, W. C. McDuffie, and
C. V. Bowen-1/26/56

4. A letter from someone in Washington should be written to Professor Robert D. Walker, Chemical Engineering Department, University of Florida for his interest and help in the odor removal problem. Without any doubt his suggestions have shortened the time towards a successful application of our ideas and data, and made the solution more economically feasible.

Remark: It was Mr. George Block from the Chemistry Section here who suggested the general use of activated charcoal.

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2. Activated charcoal is a very effective adsorbent for many organic
compounds and is used in many industrial processes. It is a very
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2.1

Activated charcoal is a very effective adsorbent for many organic
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3. A number of factors should be considered in the selection of
activated charcoal for a particular application. These factors include
the type of compound to be adsorbed, the concentration of the
solution, the pH of the solution, and the temperature of the
solution.

4. The following table gives a summary of the factors which should
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